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ARTIFACTS IN THE PROPAGATION OF STRAINS OF CHRONIC RESPIRATORY
DISEASE AGENTS AND STRAINS OF TURKEY SINUSITIS AGENTS IN VITRO

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The reports of Delaplane (1943, 1949), Jerstad and Hamilton (1948), Hitchner (1949), Hoyt et al (1951), and others concerning the propagation of the agents of chronic respiratory disease (CRD) and turkey sinusitis (TS) in chicken embryos suggested that these agents probably were viruses. More recently, the investigations of Markham and Wong (1952), Grumbles et al (1953), and Adler (1953), and in retrospect the earlier work of Nelson (1939), show that these agents are pleuro-pneumonia-like organisms (PPLO) which will propagate in artificial media.

The results of an exploration into the possibilities of culturing these agents on solid media is herewith reported. At the inception of this work it was thought that if a suitable technique could be developed it would be a valuable aid in the diagnosis of CRD and TS with special application for the examination of live virus, chicken embryo-propagated, vaccines for possible contamination with such agents.

The basic media employed were Difco PPLO broth and PPLO agar which are essentially beef heart infusion plus peptone. Horse serum employed at a final concentration of 20 per cent was added to each as an enrichment ingredient. The stock cultures which were used were three strains of CRD and three strains of TS, all chicken embryo-propagated.

found, however, that these structures still occurred on media prepared with serum which was held in a 60° C. water bath for 30 minutes.

At this point references concerning pseudo-pleuropneumonia-like colonies came to the authors' attention. Brown et al (1940) and Iaidlaw (1925) described artifacts which were believed to be related to the high serum content of the media and were considered to be soap crystals. They stained poorly with Giemsa stain but had an affinity for fat stain. The principal evidence that these structures were non-living was that they appeared on media containing as high as 5 per cent formalin and "regenerated" from broth cultures autoclaved for 30 minutes. Brown et al suggested that the reason these structures appeared to propagate was due to fragments of crystals serving as "seed" for the formation of new crystals. They further pointed out that the surface of agar during the process of streaking is disturbed and this also promotes the formation of the pseudo-colony.

This undoubtedly was the structure which was occurring in the current study. The behavior of the "rough" structures closely resembled in appearance, artificial transmission, and tinctorial characteristics the known facts concerning pseudo-PPLO colonies. The experiences described in this paper serve to emphasize the caution that must be used in interpreting findings relative to the pleuropneumonia group of organisms.

In the initial attempts at propagation of these cultures, examination of the agar plates after 5 to 10 days incubation at 37.5°C. revealed two distinct types of colony-like structures which were conveniently referred to as "smooth" and "rough" colonies. The first was a minute raised circular colony about 100 microns in diameter with raised center. It had somewhat the appearance of a target, the same as the illustration given by Merchant (1950) for bovine pleuropneumonia. The second was of a similar dimension and at low magnifications appeared as a tiny crater on the surface of the agar. Magnification of 100X or higher revealed a filamentous colony composed of one or more central refractile bodies with radiating beaded filaments. When "smooth" colonies were transferred they appeared to change to "rough" form. The rough forms, when transferred from agar to agar, or transferred from agar to broth, incubated for 48 hours, and then streaked on agar, invariably gave the impression that multiplication occurred. Such cultures were easily maintained and were recoverable from broth containing as high as 25 mg/cc of streptomycin.

Considerable doubt as to the true significance of these structures arose following their recovery on controls of several laboratory procedures. Then it was discovered that "rough" forms existed on some of the uninoculated agar which had been held at room temperature for several weeks. At the moment it was suspected that the serum portion of the media might be contaminated with a PPLO and that it was passing the Mandler filter which was used to render the serum sterile. It was

References

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Lantern Slides

<u>Slide No.</u>		<u>Width of Field</u>	<u>Power</u>
1	Distribution of pseudo-colonies along streak marks	3/4"	6X
2	Enlargement of #1	1/2"	13X
3	Enlargement of #1 and #2	1/3"	30X
4	Pseudo-colonies showing radiating filaments	1 mm	100X
5	Enlargement of #4. Colonies have numerous refractile central bodies.	0.5 mm	200X
6	Pseudo-colonies showing typical arrangement of radiating filaments.	1 mm	100X
7	Pseudo-colonies stained with fat stain (oil red O) counter stained with methylene blue stain.	1 mm	100X
8	Enlargement of #7.	0.5 mm	200X
9	Colony of CRD stained with Giemsa stain.	1 mm	100X
10	Pseudo colony stained with Giemsa stain	1 mm	100X

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